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Gas Chromatography

C. Bicchi, University of Turin, Turin, Italy

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An essential oil is internationally defined as the product obtained by steam distillation, hydrodistillation or expression (for citrus fruits) of a plant or of a part of it. This definition is now less strictly applied, and the fractions resulting from several other techniques that sample the volatile fraction of a plant are now erroneously classified as essential oils. In general it would be more correct to call them volatile fractions of a vegetable matrix, and to use the term essential oil more specifically for samples obtained by distillation or expression. In addition to distillation or expression, the volatile fraction of a vegetable matrix can be obtained through static or dynamic headspace gas chromatography (HS-GC), solid-phase microextraction (SPME-GC), simultaneous distillation–extraction (SDE), solvent extraction or supercritical fluid extraction (SFE).

Components of an essential oil are generally medium-to-highly volatile with medium-to-low polarity, and as a consequence GC is the technique of choice for their analysis. **Figure 1** shows the structure of some typical essential oil components. These characteristics also facilitate their identification, which in general can be achieved by combining chromatographic (retention indices) data with mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy (GC-FTIR).

This article aims to cover the main aspects related to the analysis of essential oils, in particular with sample preparation techniques related to GC; GC separation of enantiomers; multidimensional GC; identification of essential oil components through GC and/or combined techniques (GC-MS, GC-FTIR); GC-Isotope ratio mass spectrometry and authenticity of an essential oil; GC-sniffing for sensory evaluation; and statistical analysis applied to GC profiles.

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Sample Preparation

Steam Distillation and Hydrodistillation

An essential oil is classically obtained by steam or hydrodistillation via equipment based on the circulatory distillation apparatus introduced by Clevenger in 1928. Apparatus and operation modes are now well established. Several pharmacopoeias give diagrams and instructions of how to obtain essential oils. **Figure 2** is taken from the *European Pharmacopoeia*.

On the other hand, sampling techniques for the volatile material are under constant evolution. The most used techniques are static or dynamic HS-GC, SPME/GC, SDE and SFE.

Headspace Sampling (HS-GC)

Static HS-GC, dynamic HS-GC HS is a sampling technique applied to the determination of volatiles in the gaseous phase in equilibrium with the matrix to be sampled.

HS-GC sampling is generally classified as static or dynamic HS. In static HS-GC, the analyte is sampled from a hermetically sealed vial after the matrix has reached equilibrium with its vapour at a predetermined temperature. **Figure 3**A shows the static HS-GC pattern of a sage sample. The sample was

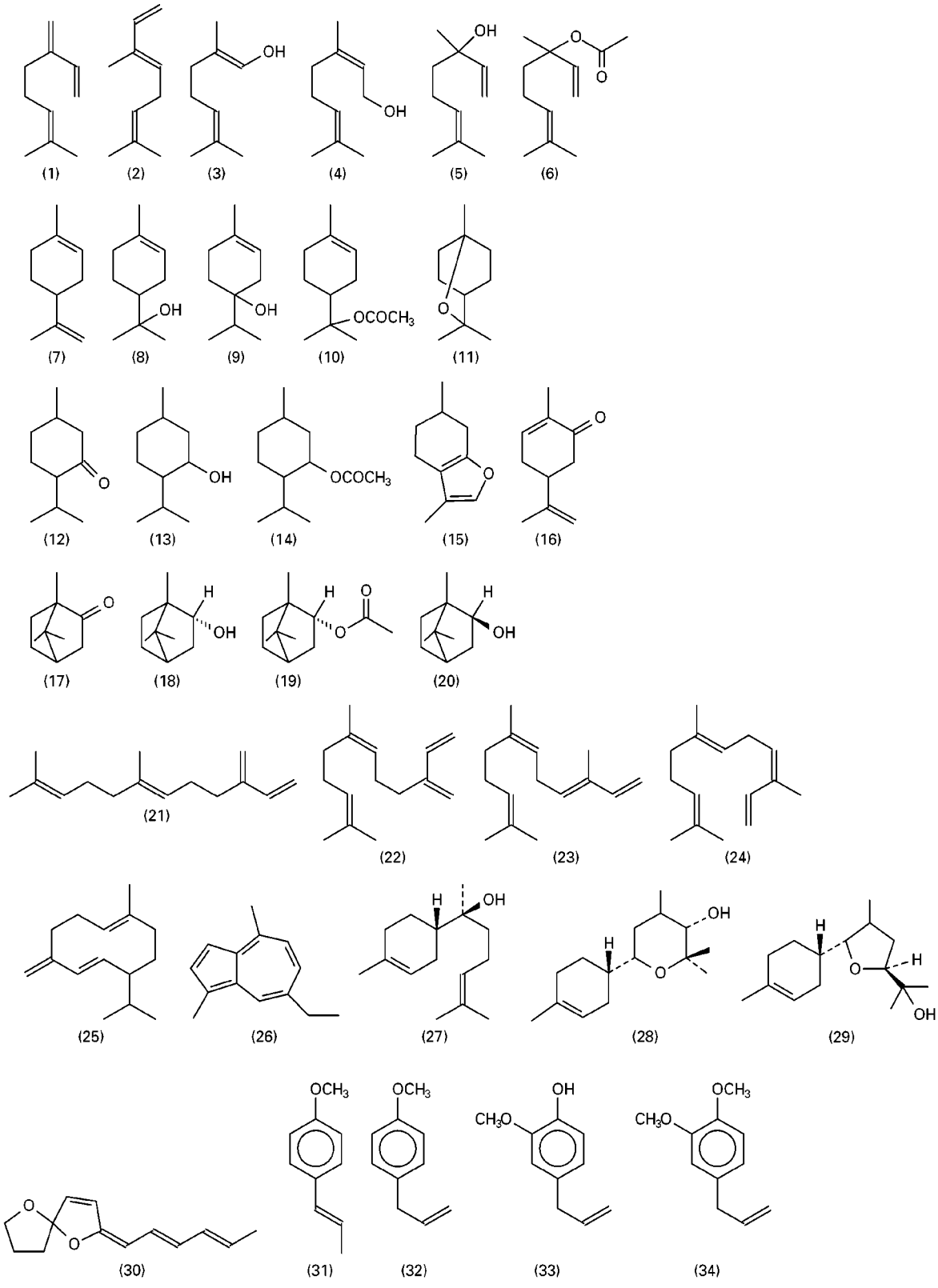

Figure 1 Structure of some typical essential oil components. 1, Myrcene; 2, *t*-ocimene; 3, geraniol; 4, nerol; 5, linalol; 6, linalyl acetate; 7, limonene; 8, α-terpineol; 9, terpinen-4-ol; 10, terpinyl acetate; 11, 1,8-cineole; 12, menthone; 13, menthol; 14, menthyl acetate; 15, menthofurane; 16, carvone; 17, camphor; 18, borneol; 19, bornyl acetate; 20, *i*-borneol; 21, *t*-β-farnesene; 22, (*Z*,*Z*)-α-farnesene; 23, (*Z*,*E*)-α-farnesene; 24, (*E*,*Z*)-α-farnesene; 25, germacrene D; 26, chamazulene; 27, (−)-α-bisabolol; 28, bisabolol oxide A; 29, bisabolol oxide B; 30, spiroether; 31, anethole; 32, estragole; 33, eugenol; 34, methyl eugenol.

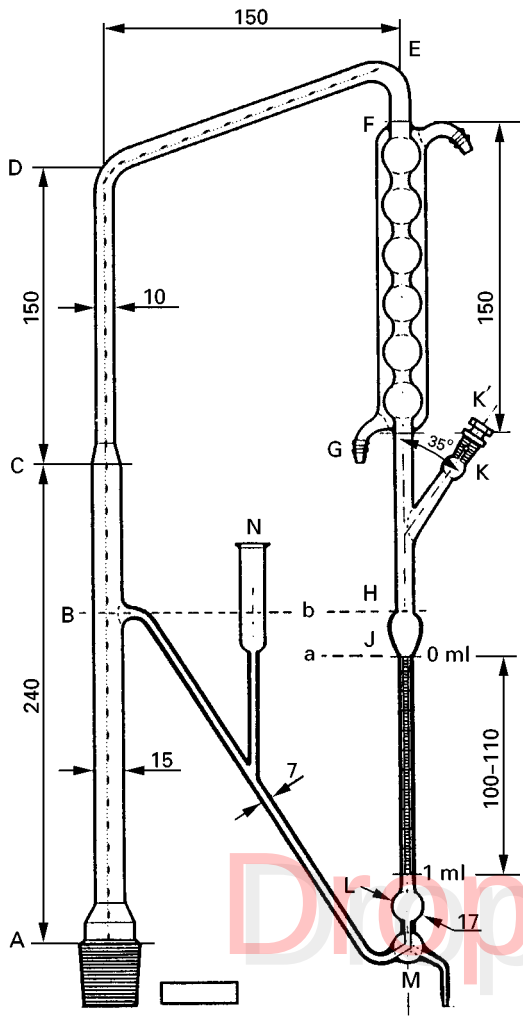

Figure 2 Apparatus for the determination of essential oils in vegetable drugs (*European Pharmacopoeia* (2000) 3rd edn, Copyright Council of Europe). Plant material suspended in water is heated to boiling; the resulting vapour, consisting of a homogeneous mixture of essential oil and steam, is then condensed in the refrigerator (F,G) and recovered in the collecting bubble (J); two layers are formed, the upper with the essential oil and the lower with the aqueous phase, the latter being continuously recirculated through the (M–B) tubing.

equilibrated for 1 h at 60°C and 1 mL of the gas phase in equilibrium with the vegetable matrix was automatically injected and analysed by GC.

In dynamic HS-GC, the sample is obtained by capturing the volatiles in a gaseous effluent passed through or over the matrix on to a suitable trapping system, such as cryotraps, solid adsorbents, liquid stationary phases or selective reagents for a given class (or classes) of compounds, coated on a solid support. The trapped volatiles are then recovered through heat or solvent elution either on-line or off-line to the gas chromatograph. **Figure 3**B shows the GC pattern of the same sage sample as in **Figure 3**A. The volatile fraction was transferred to a 50 mg Tenax TA cartridge through a nitrogen flowstream of 30 mL min^{-1} for 2 min.

Solid-phase microextraction SPME is a sampling technique based on absorption developed by Arthur and Pawliszyn. With SPME, the analytes are absorbed from the liquid or gaseous sample on to an absorbent coated fused silica fibre, which is part of the syringe needle, for a fixed time. The fibre is then inserted directly into a GC injection port for thermal desorption. SPME is a solvent-free technique which is sensitive because of the concentration factor achieved by the fibre, and selective because of the different coating materials which can be used. One of the advantages of SPME is the possibility to sample directly the vapour phase in equilibrium with the matrix (headspace (HS)-SPME), or the matrix extract or solution (liquid sampling-SPME) directly, provided that suitable fibres are used. **Figure 3**C shows the SPME-GC pattern of the same sage sample already analysed. The dried sage leaves are equilibrated as for static headspace sampling for 1 hour with a 100 μm polymethylsiloxane-coated fibre.

All these headspace techniques are easy to automate and to standardize. This is particularly true for static HS-GC and SPME-GC which can be used for fully automatic routine analysis. Static HS-GC is highly reliable for quantitative analysis, when associated with the multiple headspace extraction method developed by Kolb. Dynamic HS-GC is also quite easy to standardize, now that automatic purge-and-trap systems are commercially available. However, reproducible dynamic HS sampling is conditioned by a large number of parameters (volume to be sampled, volume of the headspace system, sampling time and speed, carrier flow rate, trapping material, including batch and producer, kinetics of component release in different matrices) that make it quite difficult to compare results from different laboratories.

The different HS sampling techniques are normally used for different applications: in general, static HS-GC is suitable for the analysis of highly volatile fractions, HS SPME-GC is suitable for the analysis of medium-volatile fractions, while dynamic HS-GC is used for trace analysis or for very diluted headspace.

Supercritical Fluid Extraction

The high selectivity of supercritical fluids, together with the low polarity and molecular weight of most of the volatile fraction components, permits low extraction pressure and temperature to be used, thus limiting the classes of the extracted components to those that characterize an essential oil (mono- and sesqui–terpenoids, phenylpropanoids and aliphatic oxygenated compounds). This often makes the com-

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position of SFE extracts quite similar to that of the corresponding essential oil obtained through hydro- or steam distillation. In addition, several organoleptically important components that are water-soluble and which are generally lost in the water phase during the steam distillation are quantitatively recovered by SFE. Typical is the case of phenylethanol, which is the main component in a rose SFE extract, while it is a minor component in the corresponding essential oil.

GC Analysis

Classical GC Analysis

Essential oils are generally analysed by capillary GC. The most popular stationary phases used for essential oil analysis are methylpolysiloxanes (SE-30, OV-1, OV-101, DB-1, PS-347.5) and methylphenylpolysiloxanes (SE-52, SE-54, PS-086, DB-5) as apolar stationary phases; and polyethylene glycol

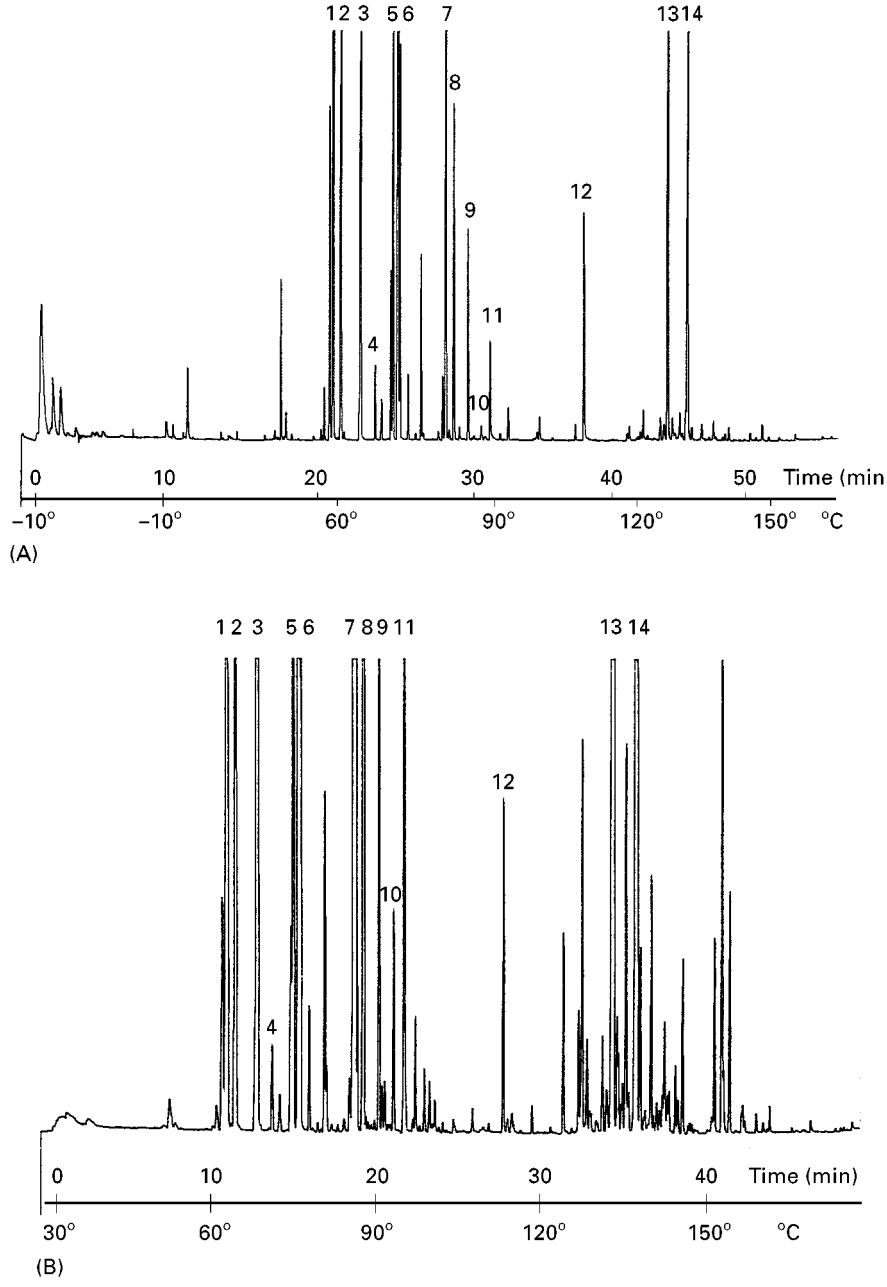

Figure 3 Capillary GC patterns of (A) the static HS-GC; (B) dynamic HS-GC and (C) HS SPME-GC of a sample of dried sage leaves. Analysis conditions: column 15 m, 0.25 mm i.d. OV-1, df: 0.3 μm; temperature programme: (A) from −10°C (10 min) to 30°C at 30°C min^{-1} then to 150°C at 3°C min^{-1} and to 200°C at 5°C min^{-1}; (B) and (C) from 30°C to 150°C at 3°C min^{-1} and to 200°C at 5°C min^{-1}. Peak identification: 1, α-pinene; 2, camphene; 3, β-pinene; 4, myrcene; 5, limonene; 6, 1,8-cineole; 7, α-thujone; 8, β-thujone; 9, camphor; 10, *iso*-borneol; 11, borneol; 12, bornyl acetate; 13, β-caryophyllene; 14, α-humulene.

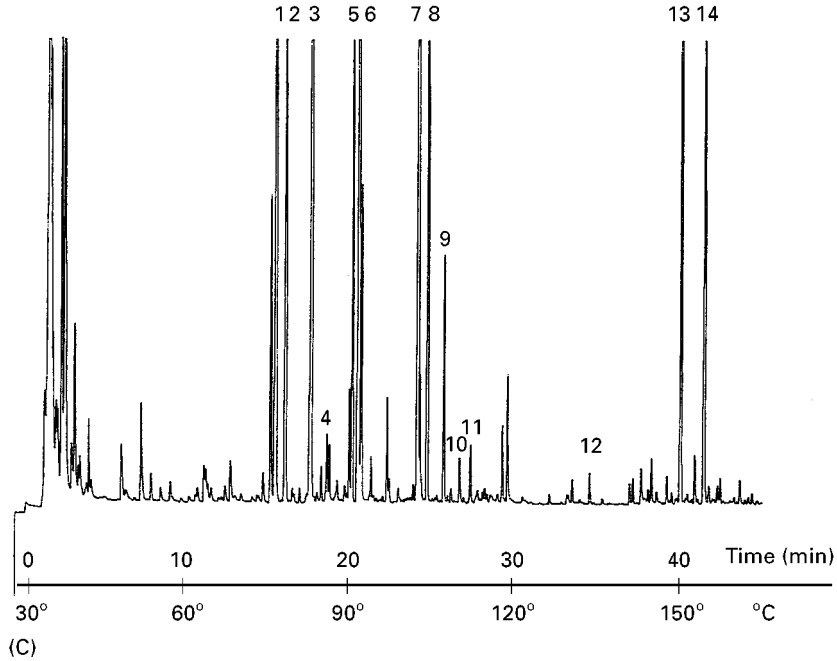

Figure 3 *Continued*

(Carbowax 20M) as a polar stationary phase. GC data are also very useful to identify most of the components in an essential oil: an effective approach is to combine the retention data from two different-polarity stationary phases (see below).

Enantiomer GC Analysis

One of the most important successes of the last 10 years has been enantioselective GC recognition of chiral essential oil components with cyclodextrin derivatives (CDs). The importance of enantiomer separation and of determining enantiomer excess is well known. Biosynthetic and geographical origins, as well as technological treatments and/or authenticity of most of the essential oils, can now also be evaluated through the enantiomeric composition of their underivatized optically active components. This is also important because optical isomers can have different sensory properties, such as the well-known cases of the different smells of both carvone and limonene enantiomers. The first GC separations of enantiomers through CDs were obtained by Koscielski and Sibilska in 1983; they separated α- and β-pinene, the corresponding pinanes and Δ-3-carene with a column packed with underivatized α-CDs. The first capillary column applications were in 1987 with the almost contemporary work of Juvancz and Schurig. CDs are generally carried in apolar to moderately polar polysiloxanes, as first proposed by Schurig. The chief reasons for this are the wider range of operating temperatures; the inertness and efficiency of columns prepared by high temperature silylation; the possibilities of tuning column polarity by using different diluting phases; the small CD amounts necessary to prepare columns; shorter analysis times; and the possibility of measuring the thermodynamic parameters involved in enantiomer discrimination.

Almost all the essential oil components can now be separated on CD stationary phases without derivatization. This is particularly true for the so-called second-generation CDs, developed especially for GC, which show high enantioselectivity, and afford highly reliable column performance. The most successful CDs are symmetrically and asymmetrically alkylated CDs, acylated CDs and CDs asymmetrically substituted in position 6 with the groups *tert*-butyldimethylsilyl or thexyldimethylsilyl, and in positions 2 and 3 with methyl, ethyl or acetyl groups. In general, the most popular matrices for the CDs are polyphenylcyanospropylsiloxes (including various OV-1701 types), polyphenylsiloxanes (including SE-52 or PS-086) and methyl polysiloxanes (including SE-30, OV-1 and PS-347.5). The latest generation of CDs makes it possible to characterize an essential oil by determining the enantiomer abundances of several of its optically active components simultaneously, very often in a single GC run. **Figure 4** shows the simultaneous enantiomer separation of optically active components characterizing lavender oil: linalyl oxides, linalol, linalyl acetate, camphor, borneol, bornyl acetate, α-terpineol and *cis*- and *trans*-nerolidols are successfully and simultaneously

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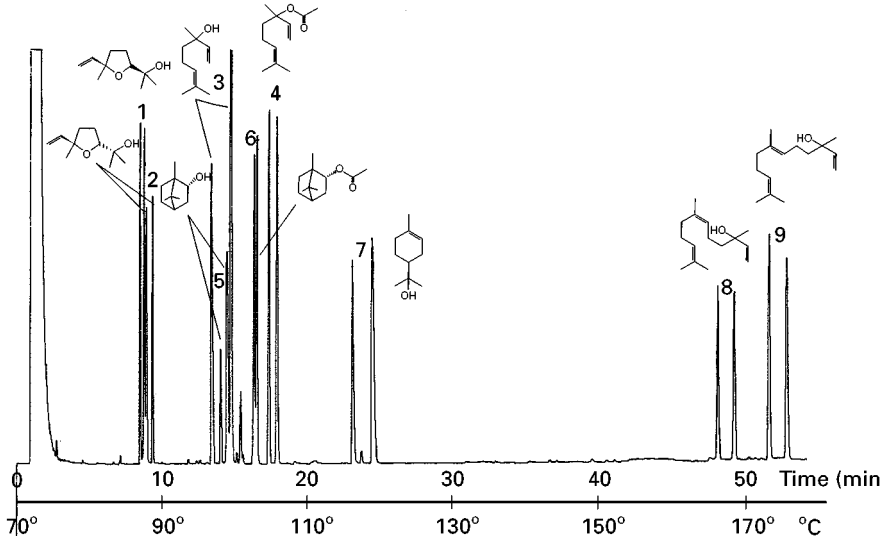

Figure 4 Simultaneous enantiomer GC separation of optically active components characterizing lavender oil: 1, *cis*-linalyl oxide; 2, *trans*-linalyl oxide; 3, linalol; 4, linalyl acetate; 5, borneol; 6, bornyl acetate; 7, α-terpineol; 8, *cis*-nerolidol; 9, *trans*-nerolidol. Column: 25 m, 0.25 mm 30% 2,3-diethyl-6-*t*-butyl-dimethylsilyl-β-CD/PS-086, film thickness 0.15 μm. Analysis conditions: from 70°C (1 min) to 190°C (10 min) at 2°C min^{-1}.

separated with a 30% 2,3-diethyl-6-*t*-butyl-dimethylsilyl-β-CD in PS-086.

Koenig and Joulain have made a very important contribution to this field: they have identified and structurally characterized about 330 sesquiterpene hydrocarbons, including the enantiomer recognition of the optically active ones. **Figure 5** shows the enantioselective GC pattern of a group of sesquiterpene hydrocarbons (δ-elemene, α-copaene, *ar*-curcumene, β-bisabolene and (*E*)-α-bisabolene) separated on a 20% 2,6-di-*O*-methyl-3-*O*-pentyl-β-CD/OV-1701 column.

Multidimensional GC

Multidimensional GC (MDGC) is a very useful technique to analyse a complex mixture such as an essential oil. In MDGC, groups of components not separated on the first column can automatically be transferred on-line to a second column coated with a different stationary phase. The possibilities of MDGC are still not fully appreciated: it is true that early systems were difficult to tune, inflexible and above all very expensive; however, most of the present systems

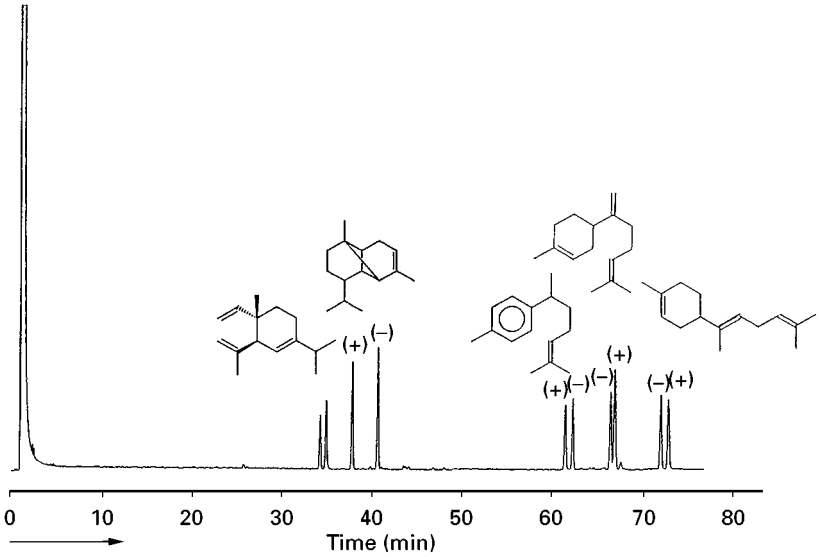

Figure 5 Enantioselective GC pattern of a δ-elemene, α-copaene, *ar*-curcumene, β-bisabolene and (*E*)-α-bisabolene separated on a 20% 2,6-di-*O*-methyl-3-*O*-pentyl-β-CD/OV-1701 column. Analysis conditions: from 60°C (1 min) to 190°C (10 min) at 0.6°C min^{-1}. (Courtesy of Professor W. Koenig, University of Hamburg.)

are fully automatic and not too expensive. Above all, they consist of two independent GC units connected through a transfer interface, which can be used independently when MDGC is not necessary.

MDGC is particularly useful with enantiomer GC analysis, which may double the number of peaks of the optically active components, making the chromatogram resulting from the analysis of an essential oil even more complex, and increasing the probability of peak overlap, thus interfering with a correct determination of enantiomeric ratios. In these cases MDGC operates a sort of clean-up on the first column, so that only selected peaks are transferred to the chiral column.

Identification

Essential oil components are generally identified through GC or GC-MS or, better, through their combination. The safest way to identify an essential oil component, and in particular a sesquiterpene, is to combine dual-column GC data and mass spectrometry (MS) data with IR data, because of the high structure-related specificity of infrared spectroscopy (IR) signals. It is important to remember that identification and structure elucidation are totally different things: identification can only be by comparison with reference data. It is risky to propose a new structure or, worse, a new skeleton, from results obtained only by combined techniques (GC, GC-MS, GC-FTIR) without parallel isolation and spectroscopic investigation (in particular, nuclear magnetic resonance) of the new compound.

As in all fields of analytical chemistry, the introduction of data systems has revolutionized the approach to identification. Operators can now build their own personal libraries with retention indices and mass spectra obtained with their own instruments, and combine the GC and GC-MS data for crossed identification of essential oil components.

Identification through Chromatographic Data

Since essential oils are generally very complex mixtures, reliable GC location and identification of their components can only be through retention indices, calculated by the Kováts method or with the van den Dool algorithm; these make retention values independent of GC conditions. Identification through retention indices is in general only considered significant when two successful matches are obtained from different-polarity stationary phases. When a suitable reference database is available, the percentage of correct identifications obtained through retention data is generally around 65% with one column, 80% with two different-polarity columns, and above 90% with three columns. The last two percentages are close to that obtainable with MS, which is generally around 90%. Since a GC system affording simultaneous injection into two columns is simple to assemble, and today's processing systems can easily handle two detector signals, a manual or automatic crossed-identification procedure is not difficult to set up. This is particularly true with the latest-generation instruments: the development of GC instruments with electronic pressure control of the mobile phase and of GC ovens in which the temperature is strictly controlled and evenly distributed, has overcome several problems with instrumentation. These give rigorous control of mobile-phase parameters (flow rate, pressure and average linear velocity) and of temperature parameters over the whole GC run. Thus the chromatographic process, and hence retention, becomes highly reproducible: as an example, under fixed conditions a retention index precision of 1 unit was maintained over 1 month for some of the most significant essential oil components of both *Matricaria chamomilla* (OV-1 column) and *Tagetes lucida* (CW-20M column; **Table 1**).

Retention indices are fundamental in making retention a reliable identification tool for GC, although many problems still exist; in particular, the variation of stationary-phase polarity and mobile-phase characteristics as a function of temperature in programmed analysis. As a consequence, a comparison of data from different laboratories can only be made with analyses run under carefully controlled operating conditions.

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Identification by GC-MS

Mass spectral data are often – and perhaps to some extent erroneously – considered the key for component identification. Many people give too much priority to mass spectrometry (MS) data over chromatographic data, and seldom give due weight to the complementarity of GC and MS data. This is probably because many manufacturers and operators do not yet consider GC-MS as a technique in its own right, but a simple coupling between GC and MS.

Nowadays, identification is generally made through commercially available mass spectra libraries (NBS, NIST, Wiley, TNO); these are nonspecialized collections of spectra mainly taken from the literature. As a consequence, the identification of a component must be carefully confirmed, since the mass spectra are from different origins and have been recorded under different operative conditions. A classical example is the differences in the spectra produced by different mass analysers: ion trap, quadrupole or magnetic sector instruments. Most operators overcome this problem by building dedicated libraries

Table 1 Reproductivity over time of reference index of *Matricaria chamomilla* L. essential oil component (OV-1) and of *Tagetes lucida* Cav. essential oil components (CW-20M)

Matricaria chamomilla *L*				Tangetes lucida *Cav*			
	Compound	*RI*[a]	*RI*[b]		*Compound*	*RI*[a]	*RI*[b]
1	*Trans*-β-farnesene	1442	1441	1	Myrcene	1159	1157
2	Bisabolol oxide B	1619	1618	2	*Trans*-β-ocimene	1247	1246
3	α-Bisabolone oxide A	1637	1637	3	Linalol	1553	1553
4	α-Bisabolol	1649	1649	4	Estragole	1656	1656
5	Chamazulene	1674	1674	5	Anethole	1807	1805
6	Bisabolol oxide A	1702	1701	6	Methyl eugenol	2006	2005
7	Spiroether	1805	1805	7	β-Caryophyllene	1566	1566
				8	Germacrene D	1675	1673

RI[a] Reference initial index; RI[b] Reference index calculated 1 month later under the same conditions. GC analysis: columns: 25 m, 0.25 mm i.d. OV-1 column, df: 0.3 μm; 25 m, 0.25 mm i.d. CW-20 m column, df: 0.25 μm. Analysis conditions: injection: split, split ratio 1 : 20, temperature 230°C; detector: FID, temperature 250°C; temperature programme: from 50°C (1 min) to 220°C (10 min) at 3°C min^{-1}; carrier gas: hydrogen, constant flow: 1.5 mL min^{-1}.

consisting of spectra recorded with their own GC-MS systems. Libraries dedicated to the essential oil field are also available, as is the case of Adams library for ion trap mass spectra, or the Joulain and Koenig collection of sesquiterpene hydrocarbon spectral data.

Chromatographic data used either actively or passively in a library search can play a fundamental role in the successful identification of essential oil components. Several compounds, in particular sesquiterpenoids, have low resolution mass spectra that are almost indistinguishable. In this case, mass spectra can mainly be used to locate the spectra in the total chromatogram; retention indices (better if from two different polarity columns) are then used to identify each component. **Figure 6** shows retention indices and mass spectra of *cis* and *trans*-α-irones.

The use of retention indices, as a further active identification key in combination with mass spectra or within the classical library search procedure, can be extremely useful. The ideal procedure should include simultaneous and/or sequential searches with retention indices from two different stationary phases, and mass spectra in which flexible and selectable priorities can be actuated.

Unfortunately, identification by retention indices associated with mass spectra is not absolutely risk-

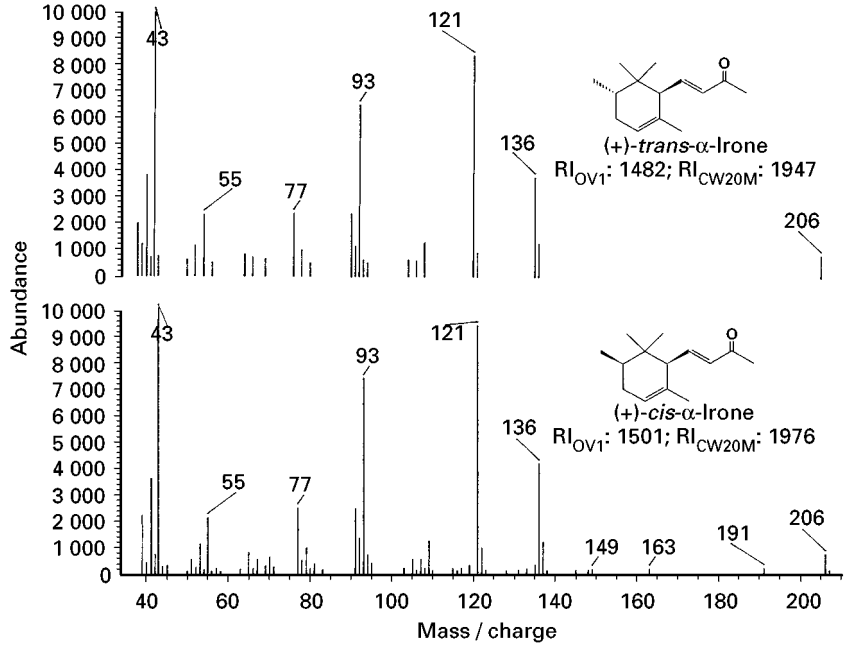

Figure 6 Retention indices on OV-1 and CW-20M and mass spectra of *cis* and *trans*-α-irones.

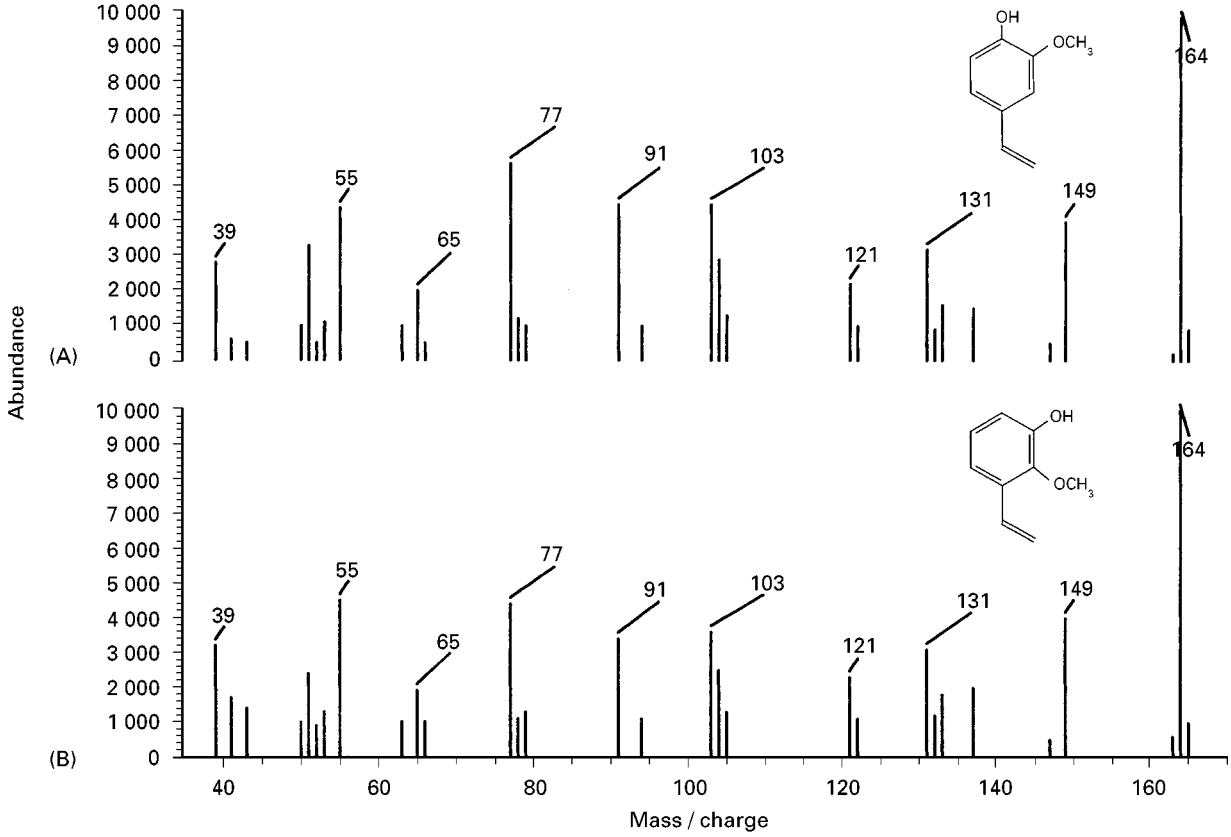

Figure 7 Retention indices on OV-101 and DB-Wax and mass spectra of (A) eugenol (RI(OV-101): 1323; RI(DB-Wax: 2158) and (B) 2-methoxy-3-hydroxy-allylbenzene (RI(OV-101): 1325; RI(DB-Wax): 2160). (Courtesy of Professor K.-H. Kubeczka, University of Hamburg.)

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free: there are some rare exceptions in which pairs of compounds have almost identical mass spectra, and also have retention indices which fall within the instrumental and analytical limit on two different stationary phases. This is the case of mass spectra and retention indices of 2-methoxy-3-hydroxy-allylbenzene and eugenol on OV-101 and DB-Wax (**Figure 7**).

A last approach, no less important, is GC/single ion monitoring (SIM)-MS, which is very selective and as a consequence the most reliable procedure for quantitative analysis. In particular, GC-SIM-MS can be an alternative to MDGC for direct determination of enantiomeric ratios of optically active components in the essential oil. A suitable choice of specific diagnostic ions can overcome the interferences to a correct determination of enantiomeric ratio due to peaks coeluting with the two (or more) enantiomers.

Identification by GC-FTIR

Although MS is the preeminent technique to identify a component in a complex mixture analysed by GC, it has some drawbacks, e.g. in the differentiation of structural isomers giving identical mass spectra. Fourier transform infrared spectroscopy (FTIR) combined with GC has emerged as a powerful technique and as the ideal complement to MS for component identification in complex mixtures, thanks to its ability to distinguish geometric and positional isomers and to characterize organic functions; moreover, the identification of a compound through its FTIR spectrum is very reliable. To exploit in full the complementarity between FTIR and MS data, systems combining online GC, FTIR and MS or FID have also been assembled. In spite of this, FTIR, as a detector for GC, is not as popular as MS, because of the lack of sensitivity for many compounds when compared to GC or GC-MS systems. Another reason is the lack of extensive libraries. The cheapest and most widely adopted GC-FTIR system is based on the so-called light–pipe interface, which produces vapour-phase spectra: this makes existing collections of spectra useless for automatic identification, because they are mainly recorded in the liquid or solid phase. Some relatively small collections of vapour-phase spectra of compounds in the essential oils and flavour fields are now commercially available.

GC-Isotope Ratio Mass Spectrometry

The stable isotope ratio is an important parameter in biochemistry, nutrition and drug research, and in origin assignment and authenticity control of essential oils. This ratio has gained in importance with the introduction of on-line coupled GC-isotope ratio MS systems, where the analytes eluting from the GC column are combusted to carbon dioxide in an oven and analysed in an isotope ratio mass spectrometer, adjusted for the simultaneous determination of mass 44 ($^{12}C^{16}O_2$), 45 ($^{13}C^{16}O_2$, $^{12}C^{16}O^{17}O$) and 46 ($^{12}C^{16}O^{18}O$) in the nmol range and with high precision ($\leq 0.3‰$). The actual ratio is obtained from the ratio of the areas of two isotope peaks; this value is then compared to a standard value by applying the following expression:

$$\delta = (R_{sa}/R_{st} - 1) \times 1000$$

where R_{sa} is the isotope ratio of the sample and R_{st} that of the standard; δ-C^{13} is given in parts per thousand.

The δ-C^{13} value is particularly effective when combined with enantiomeric recognition of the chiral component(s) characterizing an essential oil. Enantiomer GC analysis may fail in authenticity determination when recemates of natural origin are present, or when racemization occurs during processing or storage of natural products, and chiral essential oil components are blended with the corresponding synthetic chiral compounds.

Enantiomeric GC separation combined with isotope ratio MS is a very effective method of evaluating the authenticity of an essential oil since the mass spectrometer detects enantiomers of the same natural source which have identical δ-C^{13} values. As a consequence, identical δ-C^{13} ratios are expected for enantiomers from genuine compounds, even if the chiral molecules to be analysed are partially racemized: it seems improbable that racemic compounds would be synthesized through different biochemical pathways in the same organism. Enantiomer GC-isotope ratio MS or, even better, multidimensional GC-isotope ratio MS of enantiomers can therefore detect blends of optically pure chiral essential oil components with synthetic racemates.

Sensory Analysis (GC-Sniffing Detection)

The GC profile of an essential oil does not necessarily reflect the sensory properties of its components. Some of the components, present in large quantities, are not relevant to the overall smell while others, present in trace amounts but with low detection thresholds, are not revealed by FID, nitrogen–phosphorous detector (NPD) or flame photometric detector (FPD) but are detected by GC-sniffing. Sensory methods are therefore needed to detect trace components responsible for the smell of an essential oil. A sniffing device is very simple and inexpensive to assemble. Several have been described: in a typical one, the flow of the gas eluting from the analytical column is split through a T piece on one side to an FID and on the other to the sniffing port, which consists of a shaped glass funnel. A stream of air (or nitrogen) saturated with water is sent coaxially with the mobile phase to the sniffing port to avoid dehydration of the nasal tissues. The results of olfactory measurements can be qualitative, giving a description of the odour of each peak corresponding to an odour-active component; on the basis of these qualitative results, a semiquantitative evaluation is also possible. Several approaches have been developed for semiquantitative sensory evaluation: the best known are Charm analysis developed by Accree and AEDA (aroma extract dilution analysis), developed by Grosch. Charm analysis is based on sniffing a series of decreasing dilutions of the components eluting in the GC odour-active zones characterized with a specific sensorial descriptor. The beginning and end of each particular odour perception are fixed. Charm values are calculated through the formula $c = d^{n-1}$, where n is the number of coincident responses and d is the dilution factor. AEDA is similar but it uses a dilution factor equal to the last dilution in which an odour-active component is detected.

GC Profile Analysis

Identification and quantitation of the characterizing components are not always sufficient to discriminate between essential oils from a single species, or to classify them, evaluate their quality or origin, or detect adulterations. On the other hand, although sensory analysis (sniffing) is of prime importance in overall evaluation, it is also sometimes insufficient and, above all, it is generally considered insufficiently objective. This is particularly true when series of samples of different origins have to be evaluated at the same time. In these cases, the overall GC profiles of the essential oils under investigation (or the profile of the volatile fraction obtained through related sampling techniques) can be a successful marker to characterize and/or discriminate between them objectively. However, essential oil profiles are so complex that multivariate statistics is needed to obtain an exhaustive evaluation. Several statistical approaches have

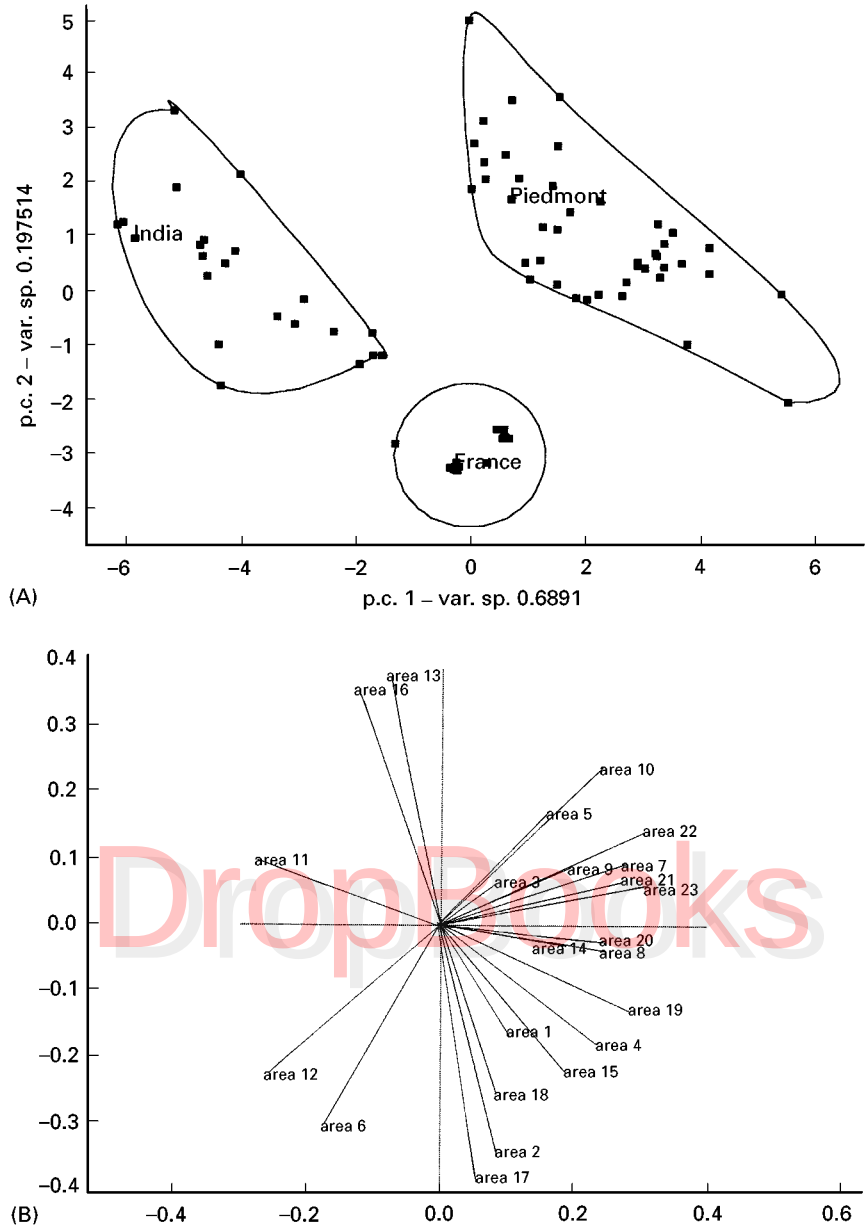

Figure 8 (A) Scatterplot of principal components and (B) distrbution of the loadings considered for PCA of a set of peppermint essential oils of different origins (Piedmont (Italy), France and India.)

been proposed (cluster analysis, fuzzy clustering, linear discrimination analysis, neural network, principal component analysis (PCA), and principal component similarity analysis). PCA is successful in analysing multivariate data, since it can investigate relationships between large numbers of variables, and it is useful for reducing the numbers of variables in a data set by finding linear combinations of those variables that explain most of the variability. These characteristics make PCA successful in comparing and discriminating groups of essential oils, for instance versus a series of reference samples, in particular for routine purposes. The success of PCA is strictly related to a correct selection of variables (the peak areas corresponding to specific essential oil components, generally chosen among those whose peak areas are detectable and reproducibly measurable in all samples under investigation). **Figure 8** shows the distribution of the loadings considered for PCA and the scatterplot of principal components of a set of peppermint essential oils of different origins (Piedmont (Italy), France and India): PCA clearly distinguishes the origins of the samples.

Conclusions

Although essential oil analysis is now a well-established field, further work is needed, not only to improve sample preparation and analysis techniques, but also to deal with one of the main aims of this research field: to isolate and elucidate the structure of new odorous compounds. These studies evolve along two main lines. The first and classical one combines isolation and spectroscopic techniques and mainly concerns new mono- and sesquiterpenoids. The second mainly involves the so-called supervolatile fraction and perfumed trace compounds, two fractions that play a fundamental role in odour impact. For the supervolatile fraction, some topics requiring further study are HS combined with effective cryotrapping techniques, systems for direct GC injection of large volumes of gas samples and GC columns with a high retention capacity. For compounds present in the essential oil at the p.p.m. level (e.g. pyridine derivatives in peppermint and orange oils), a number of points would benefit from further investigation. These include increased selectivity of sample preparation techniques and increased sensitivity and selectivity of analysis techniques.

See also: **II/Chromatography: Gas:** Column Technology; Detectors: Mass Spectrometry; Detectors: Selective; Headspace Gas Chromatography; Sampling Systems; **III/Essentials Oils:** Distillation. **Terpenoids: Liquid Chromatography**.

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Thin-Layer (Planar) Chromatography

P. Dugo, L. Mondello and G. Dugo, Università di Messina, Messina, Italy

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Introduction

Essential oils are mixtures of mainly volatile components belonging to different chemical classes. They